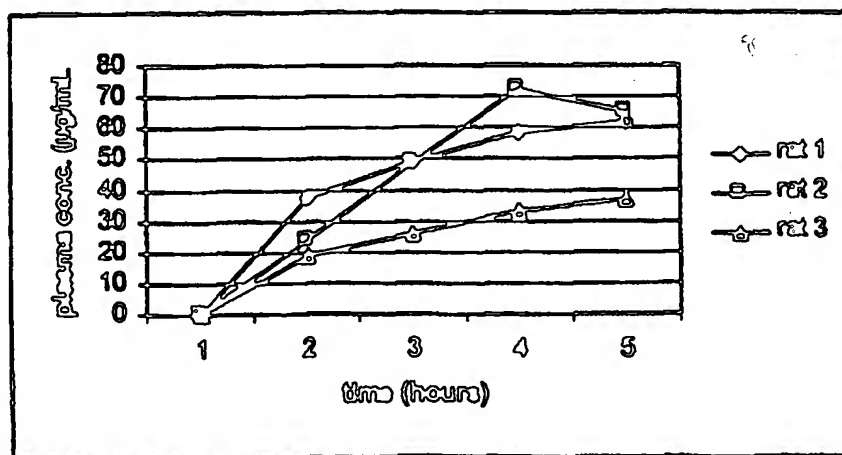




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification <sup>7</sup> :  <b>A61K 35/64, 48/00, C12Q 1/68, C07H 21/02, 21/04</b></p>	<p><b>A1</b></p>	<p>(11) International Publication Number: <b>WO 00/50050</b></p> <p>(43) International Publication Date: <b>31 August 2000 (31.08.00)</b></p>
<p>(21) International Application Number: <b>PCT/US00/04662</b></p> <p>(22) International Filing Date: <b>23 February 2000 (23.02.00)</b></p> <p>(30) Priority Data:  <b>09/256,515</b>      <b>23 February 1999 (23.02.99)</b>      <b>US</b></p> <p>(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application  <b>US</b>      Not furnished (CIP)  <b>Filed on</b>      Not furnished</p> <p>(71) Applicant (for all designated States except US): <b>ISIS PHARMACEUTICALS, INC. [US/US]; 2292 Faraday Avenue, Carlsbad, CA 92008 (US).</b></p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): <b>HARDEE, Gregory, E. [US/US]; 17407 La Brisa, Rancho Santa Fe, CA 92067 (US). TILLMAN, Lloyd, G. [US/US]; 4350 Stanford Street, Carlsbad, CA 92008 (US). MEHTA, Rahul, C. [IN/US]; 695 Shenandoah, San Marcos, CA 92069 (US). TENG, Ching-Leou [US/US]; 4571 Mercurio Street, San Diego, CA 92130 (US).</b></p>		<p>(74) Agents: <b>AXFORD, Laurie, A.; Foley &amp; Lardner, 23rd floor, 402 West Broadway, San Diego, CA 92101-3542 (US) et al.</b></p> <p>(81) Designated States: <b>AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</b></p>
		<p><b>Published</b>  <i>With international search report.</i></p>

(54) Title: MULTIPARTICULATE FORMULATION



**(57) Abstract**

The present invention is related to non-parenteral multiparticulate formulations capable of transporting therapeutic, prophylactic and diagnostic agents across mucosal membranes such as gastrointestinal, buccal, nasal, rectal and vaginal. Formulations comprise a plurality of carrier particles, an agent to be delivered across a mucosal membrane, and a penetration enhancer. The drug is adhered to the surface of the carrier particle or is impregnated within by electrostatic, covalent or mechanical forces.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	IS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

## Multiparticulate Formulation

### FIELD OF THE INVENTION

5

The present invention is related to pharmaceutical formulations, in particular non-parenteral pharmaceutical formulations capable of delivering therapeutic and diagnostic agents across mucosal membranes.

10

### BACKGROUND OF THE INVENTION

Parenteral administration of pharmaceutical agents or drugs by injection (intravenous, subcutaneous, intramuscular) while common and necessary under certain circumstances is not the most desirable route from a patient standpoint. Because it can seldom be performed by the individual in need thereof and requires assistance of professional care givers it is an inconvenient and costly route. Further, there is often associated discomfort at the site of administration and there is always an inherent risk of infection. Not surprisingly, patient compliance is much greater for drugs administered non-parenterally,

15

20

25

in particular, oral, nasal and pulmonary administration. For their convenience and non-invasive nature, these non-parenteral routes of administering drugs are preferred by patients. However, each of  
5 these involves transport of the drug across a mucosal surface or membrane, which comprises an epithelium and a mucus secretion thereon. The mucus secretion of a mucosal membrane presents a barrier to protect the membrane from physical damage as well as to prevent  
10 undesired substances from passing through and entering epithelial tissue or the lymph system or the blood stream. Unfortunately, mucus membranes can also prevent significant uptake of some drugs such as those having large molecular weight, or are proteinaceous or  
15 are nucleic acids. Consequently these drugs are most often administered to individuals parenterally, for example, injected intravenously, subcutaneously or intramuscularly.

It would therefore be desirable to provide a  
20 convenient formulation for transporting pharmaceutical agents across mucosal membrane.

#### **SUMMARY OF THE INVENTION**

25 In accordance with an aspect of the present invention there is provided a multi-particulate formulation or composition comprising a plurality of carrier particles; a biologically active substance to  
30 be delivered across a mucosal membrane, wherein said biologically active substance is bound to said carrier particles; and a penetration enhancer.

In another aspect of the invention there is provided a method of delivering a biologically active  
35 substance across a mucosal membrane, by introducing to

the mucosal membrane a multi-particulate formulation comprising a plurality of carrier particles; the biologically active substance and a penetration enhancer, wherein said biologically active substance is bound to said carrier particles.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing concentration of antisense oligonucleotide in plasma at time intervals following administration of a multiparticulate formulation.

#### DETAILED DESCRIPTION OF THE INVENTION

A multi-particulate formulation or composition is provided having a plurality of carrier particles; a biologically active substance (BAS) to be delivered across a mucosal membrane, and a penetration enhancer, wherein said biologically active substance is bound to said carrier particles. Formulations of the invention associate with mucosal membranes such as buccal, nasal, pulmonary, gastrointestinal and vaginal, thereby transporting biologically active substances to an individuals lymph system, blood stream or epithelial tissue.

##### carrier particles

Carrier particles according to the present invention include a variety of particle-forming substances that are preferably capable of maintaining a biologically active substance (BAS) in intimate association with mucosal membranes thereby enhancing transport of the BAS across mucosal membranes.

Preferred carrier particles are those which enhance bioavailability of biologically active substances upon administration and delivery to a mucosal membrane.

"Bioavailability" in this context is the percentage of the total amount of BAS administered that is found in plasma, epithelial tissue or target tissue at a given time post administration. For enhancing

bioavailability of a BAS, it is preferred that carrier particles are composed of material that resists degradation prior to contacting a mucosal membrane.

Carrier particles, depending on their chemical composition and mode of preparation, include a variety of regular or irregular shapes and sizes and may be a solid or a gel. For example, preferred carrier particles are generally spherical (hollow or filled) having millimeter (greater than about 1 mm), micron (greater than about 1 $\mu$ ) or nanometer (greater than about 10nm) diameter and are thus referred to as

miniparticles (tablets), microparticles and nanoparticles respectively. Preferred carrier particles have a diameter of about 0.01 to 1000 $\mu$ . More preferably carrier particles are about 0.1 to 500 $\mu$  and more preferably 1 to 300 $\mu$ .

Preferred particle-forming substances include

poly-amino acids; polyimines; polyacrylates; dendrimers; polyalkylcyanoacrylates; cationized gelatins, albumins, starches, acrylates, polyethyleneglycols (PEG) and starches; polyalkylcyanoacrylates; DEAE-derivatized polyimines, pollulans, celluloses and starches;

By the term "bound" is meant that biologically active substances are associated with carrier particles by way of electrostatic (ionic, polar, Van der Waals), covalent or mechanical (non-

electrostatic, non-covalent) interaction depending on the composition of the BAS and carrier particle as well as the method of preparing the carrier particle. For example, an anionic BAS such as an oligonucleotide can be bound to cationic carrier particles by ionic interaction. In a particularly preferred embodiment, particle-forming substances are polycationic polymers such as chitosan, poly-L-lysine, polyhistidine, polyornithine, polyspermines, protamine, polyvinylpyridine, polythiodiethylamino-methylethylene P(TDAE), polyaminostyrene (e.g. para-amino), poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate, poly(isobutylcyanoacrylate), poly(isohexylcyanoacrylate), DEAE-methacrylate, DEAE-hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-dextran. In a particularly preferred embodiment, the particle-forming substance is chitosan. In another particularly preferred embodiment, the particle-forming substance is poly-L-lysine complexed with alginate. In a further embodiment, formulations of the invention comprise cationic carrier particles and anionic biologically active substances, such as oligonucleotides, associated thereto by ionic interaction.

In an alternative embodiment, particle-forming substances are non-polycationic i.e. carry an overall neutral or negative charge, such as polyacrylates, for example polyalkylacrylates (e.g. methyl, hexyl etc.), polyoxethanes, poly(DL-lactic-co-glycolic acid (PLGA), and polyethyleneglycol (PEG). In a particularly preferred embodiment, the particle forming substance is PLGA.

In another embodiment, carrier particles further comprises an outer coating. Said outer coating may be a material capable of associating a BAS to the carrier particle, for example, a cationic polymer which

binds an anionic BAS, or be a protective material  
resisting degradation in biological environments such  
as in the stomach, lumen, plasma or cytoplasm. In a  
particular embodiment, carrier particles may be  
5 substantially coated on their outer surface with lipid  
compounds as described in WO98/29,557, WO98/29,099,  
WO98/04,719, WO97/12,618 and WO92/21,330 incorporated  
herein by reference. The lipid coating serves to  
enhance cellular membrane fusion and therefor cellular  
10 uptake of particles.

Further, carrier particles may have attached  
thereto targeting molecules which serve to bind the  
particle to the mucosal membrane cells and/or to direct  
the particle once across the mucosal membrane to a  
15 particular cell, tissue or organ type of interest.  
Targeting molecules may be peptidic such as proteins or  
peptides or small molecules. Protein targeting  
molecules include antibodies which selectively bind to  
antigenic determinants that are predominant at the site  
20 of interest. Protein and peptide targeting molecules  
are preferably those that are selective ligands for  
cell surface receptors. For example, certain growth  
factors such as EGF (epidermal growth factor) and PDGF  
(platelet derived growth factor) are overexpressed on  
25 the surface of certain cancer cells. The proteins EGF  
and PDGF therefor serve as a suitable targeting  
molecule for directing carrier particles containing  
anticancer agents. More preferably, targeting  
molecules are peptide fragments of proteins which bind  
30 to cellular receptors. Similarly, certain small  
organic molecules are ligands for cell surface  
receptors. For example, folic acid receptors are known  
to be overexpressed in certain cancer cells.  
Consequently folate is a useful targeting molecule for  
35 delivering anticancer agents to cancer cells.  
Targeting molecules may be linked to carrier particles



of the invention by a linking group attached to a functional group of the carrier particle. Suitable linking groups include peptides, hydrocarbon chains such as alkyl, or other polymers. A particularly preferred linking group is polyethylene glycol (PEG) of approximately 1 to 250 repeating units, preferably about 20 to 100 repeating units and more preferably 70 to 80 repeating units.

10 biologically active substances

In accordance with present invention "biologically active substances" (BAS) include a wide variety of substances having pharmacological effect (therapeutic, prophylactic or diagnostic) in animals such as mammals, in particular humans. Types of biologically active substances which may be employed include small organic molecules, macromolecules and polymers such as peptides, proteins, monoclonal antibodies and fragments thereof, nucleic acids such as nucleosides, nucleotides, single stranded oligonucleotides (probes, antisense, ribozymes), double stranded oligonucleotides (vectors, plasmids). The present invention is particularly useful for transporting proteinaceous and oligo(ribo/deoxy)nucleic acids across mucosa. In a particular embodiment, oligonucleotides are employed in formulations of the invention, in particular single stranded oligonucleotides such as those having antisense or ribozyme activity. Oligonucleotides include those incorporating naturally-occurring structure i.e. 3'-5' phosphodiester linked ribo or deoxyribonucleosides or those incorporating non-naturally occurring features. For example, one or more backbone linkages of oligonucleotides may be other than naturally occurring phosphodiester, for example, phosphotriester, phosphorothioate, phosphorodithioate, phosphonates (H,

alkyl, aryl etc.), boranophosphate, selenophosphate, ethylene glycol, methylenemethylimino (MMI) and others.

Other backbone modifications include 2'-5' backbone linkages and those having an acyclic sugar-backbone structure such as Peptide Nucleic Acids (PNA's) wherein the sugar and phosphate components are replaced with a peptidic structure.

The sugar component of oligonucleotides may be modified to include hexoses, cyclopentyl or cyclohexyl as well as various substituents at the 2' position including halogen, alkoxy (2'-O-alkyl), alkoxyalkoxy (2'-O-alkyl-alkoxy) and derivatives thereof. Particularly preferred 2' substituents include methoxy, methoxyethoxy (MOE), aminooxyethoxy (AOE) and dimethylaminooxyethoxy (DMAOE),

Other non-natural oligonucleotide modifications include base modifications such as 5-methyl-cytosine and 2-aminoadenine and base or sugar functionalization such as cholesterol, intercalators and targeting molecules such as receptor ligands, peptides, antibodies and folic acid. Examples of specific oligonucleotides which may be employed in formulations of the present invention include:

25	ISIS-5132	TCCCG CCTGT GACAT GCATT	(SEQ ID NO:1)
	ISIS-2302	GCCCA AGCTG GCATC CGTCA	(SEQ ID NO:2)
	ISIS-2922	GCGTT TGCTC TTCTT CTTGC G	(SEQ ID NO:3)
	ISIS-3521	GTTCT CGCTG GTGAG TTTCA	(SEQ ID NO:4)
	ISIS-2503	TCCGT CATCG CTCCT CAGGG	(SEQ ID NO:5)
30	ISIS-13312	<u>GCGTT</u> <u>TGCTC</u> <u>TTCTT</u> <u>CTTGC</u> G	(SEQ ID NO:6)
	ISIS-5320	TTGGG GTT	(SEQ ID NO:7)
	ISIS-14803	GTGCT <u>CATGG</u> <u>TGCAC</u> GGTCT	(SEQ ID NO:8)
	ISIS-28089	GTGTG <u>CCAGA</u> <u>CACCC</u> <u>TATCT</u>	(SEQ ID NO:9)

wherein (i) each oligo backbone linkage is a phosphorothioate linkage and (ii) nucleosides in bold font incorporate a 2'-O-methoxyethyl modified sugar and (iii) underlined cytosine nucleosides incorporate a 5-methyl substituent on their nucleobase.

penetration enhancers

The present invention employs various penetration enhancers in order to effect the gastrointestinal delivery of biologically active substances (BAS). Penetration enhancers may be classified as belonging to one of five broad categories i) surfactants, ii) fatty acids, iii) bile salts, iv) chelating agents, and (v) non-chelating non-surfactants as described in Lee et al (*Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, p. 92).

i) surfactants: In connection with the present invention, surfactants (also known as "surface-active agents") are chemical entities which, when dissolved in an aqueous solution, reduce the surface tension of the solution or the interfacial tension between the aqueous solution and another liquid, with the result that absorption of biologically active substances through mucosa is enhanced. Surfactants include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, page 92) and perfluorochemical emulsions, such as FC-43 (Takahashi et al., *J. Pharm. Pharmacol.*, 1988, 40:252).

Bile salts and acids as well as fatty acids and salts thereof may also be considered to be surfactants.

ii) fatty acids: Various fatty acids, derivatives and salts thereof act as penetration

enhancers. Suitable fatty acids include, for example, oleic acid, lauric acid, capric acid (a.k.a. n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, 5 monoolein (a.k.a. 1-monooleoyle-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines and mono- and di-glycerides thereof and/or physiologically acceptable 10 salts thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.) (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, page 92; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, 7:1; El-Hariri et al., *J. Pharm. Pharmacol.*, 1992, 44:651). In a 15 preferred embodiment of the present invention fatty acid/salt penetrations enhancers are sodium caprate and sodium laurate.

iii) bile acid and salts: The physiological 20 roles of bile include the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (Brunton, Chapter 38 In: *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 9th Ed., Hardman et al., eds., McGraw-Hill, New York, NY, 1996, pages 25 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus, the term "bile acid" or "bile salt" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. The bile acids 30 and salts of the present invention include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium

deoxycholate), glucolic acid (sodium glucolate), glycholic acid (sodium glycocholate), glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (CDCA, sodium chenodeoxycholate), ursodeoxycholic acid (UDCA, sodium ursodeoxycholate), sodium tauro-24,25-dihydro-fusidate (STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether (POE) (Lee et al.,  
5  
10 *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, page 92; Swinyard, Chapter 39 In: *Remington's Pharmaceutical Sciences*, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, PA, 1990, pages 782-783; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, 7:1; Yamamoto et al., *J. Pharm. Exp. Ther.*, 1992, 263:25; Yamashita et al., *J. Pharm. Sci.*, 1990, 79:579). In a preferred embodiment, bile  
15 penetration enhancers are ursodeoxycholic acid (UDCA) and chenodeoxycholic acid (CDCA). In a more preferred embodiment, bile penetration enhancers are the sodium  
20 salts of UDCA and CDCA. Most preferably, the penetration enhancer of the invention is the sodium salt of UDCA.

iv) chelating agents: Chelating agents, as  
25 used in connection with the present invention, can be defined to be compounds that remove metallic ions from solution by forming complexes therewith, with the result that absorption of oligonucleotide through mucosa is enhanced. In an context wherein the  
30 biologically active substance of the invention is an oligonucleotide e.g. an antisense oligonucleotide, chelating agents also serve as inhibitors of nucleases. Most characterized DNA nucleases require a divalent

metal ion for catalysis and are thus inhibited by chelating agents (Jarrett, *J. Chromatogr.*, 1993, 618, 315). Chelating agents of the invention include but are not limited to disodium ethylenediaminetetraacetate (EDTA), citric acid, salicylates (e.g., sodium salicylate, 5-methoxysalicylate and homovanilate), *N*-acyl derivatives of collagen, laureth-9 and *N*-amino acyl derivatives of beta-diketones (enamines) (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, page 92; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, 7:1; Buur et al., *J. Control Rel.*, 1990, 14:43).

v) non-chelating non-surfactants: As used herein, non-chelating non-surfactant penetration enhancing compounds can be defined as compounds that demonstrate insignificant activity as chelating agents or as surfactants but that nonetheless enhance absorption of oligonucleotide through mucosa (Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, 7:1). This class of penetration enhancers include, for example, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-alkanone derivatives (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, page 92); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita et al., *J. Pharm. Pharmacol.*, 1987, 39:621).

Another compound having penetration enhancing qualities is Zonula occludens toxin (Zot) isolated from *Vibrio cholerae*. This protein has been shown to regulate intestinal tight junction permeability by receptor a mediated pathway (Fasano et al, *Proc Natl*

Acad Sci USA, 1991, 88(22):5242; and Gastroenterology, 1997, 112:839). Zot protein or fragments thereof capable of binding and activating this receptor may be co-administered with a BAS alone or in conjunction with carrier particle-bound biologically active substances, particularly when the BAS is an oligonucleotide.

Penetration enhancers are employed in formulations of the invention as an additional component or are incorporated within, the carrier particles. In the former context, penetration enhancers are in any suitable form e.g. powder, gel, solution etc. in which carrier particles are mixed. In the latter context, carrier particles are impregnated or have an outer coating of penetration enhancers covering a substantial portion of the carrier particle surface. Alternatively, penetration enhancers are formed into particles themselves which may be mixed with carrier particles. Regardless of dosage forms employed, it is preferred that penetration enhancers are presented to mucosal membranes prior to or concomitantly with the carrier particles. It is contemplated that penetration enhancers are released from the formulation prior to release of the BAS particle complex or alternatively the penetration enhancer is administered prior to the BAS particle complex.

In a particular embodiment, penetration enhancers useful in the present invention are mixtures of penetration enhancing compounds. For example, a particularly preferred penetration enhancer is a mixture of UDCA (and/or CDCA) with capric and lauric acids or salts thereof e.g. sodium. Such mixtures whether in the context of carrier particle systems of the present invention or otherwise are useful for enhancing the delivery of biologically active

substances across mucosal membranes, in particular intestinal mucosa. Preferred penetration enhancer mixtures comprise about 5-95% of bile acid or salt(s) UDCA and/or CDCA with 5-95% combined capric and lauric acid. Particularly preferred are mixtures of the sodium salts of UDCA, capric acid and lauric acid in a ratio of about 1:2:2 respectively.

Penetration enhancers and mixtures thereof are present in formulations of the invention in an amount of about 1-99% relative to the biologically active substance. Actual relative amounts will depend on the particular biologically active substance. For instance, when the biologically active substance is an oligonucleotide e.g. an antisense oligonucleotide, the amount of penetration enhancer or mixture employed is from about 40 to 95%, preferably 50 to 90%.

#### bioadhesive

In an embodiment of the invention formulations further comprise a bioadhesive material which serves to adhere carrier particles to a mucosal membrane. Preferably, carrier particles are themselves bioadhesive, as is the case with PLL-alginate carrier particles, or may be coated with a bioadhesive material. Such materials are well known in the formulation art, examples of which are described in WQ 85/02,092 incorporated herein by reference. Preferred bioadhesive materials include polyacrylic polymers (e.g. carbomer and derivatives of carbomer), tragacanth, polyethyleneoxide cellulose derivatives (e.g. methylcellulose, carboxymethylcellulose, hydroxypropylmethylcellulose (HPMC), hydroxyethylcellulose (HEC), hydroxypropylcellulose (HPC) and sodium carboxymethylcellulose (NaCPC)), karyagum, starch, gelatin and pectin.



mucolytic

5 In another embodiment of the invention, formulations further comprise a mucolytic substance which serves to degrade or erode mucin, in part or completely, at the site of the mucosal membrane to be traversed. Mucolytic substances are well known in the formulation art and include N-acetylcysteine, dithiothreitol, pepsin, pilocarpine, guaifenesin, 10 glyceryl guaiacolate, terpin hydrate, ammonium chloride, guattenesin, ambroxol, bromhexine, carbocysteine, domiodol, letosteine, mecysteine, mesna, sobrerol, stepronin, tiopronin and tyloxapol.

15 In another aspect of the invention there is provided a method of delivering a biologically active substance across a mucosal membrane, comprising introducing to the mucosal membrane a multi-particulate formulation according to the invention.

20 It is generally desirable to have as high a weight ratio of BAS to carrier particle as possible consistent with particle stability. The amount of pharmaceutical agent will vary depending on the nature and composition of the agent but in general will be 25 from about 1:10 to about 1:1000.

According to an aspect of the invention, a BAS is delivered across a mucosal membrane in an animal, in particular humans, by administering a 30 formulation of the invention to the animal. Administration is non-parenteral e.g. oral, rectal, enema, vaginal, buccal, sublingual, nasal or by pulmonary inhalation. The dosage form used will depend on route of administration, the type of therapeutic, 35 prophylactic or diagnostic indication. The dosage forms include solutions, suspensions, emulsions,

ointments, gels, tablets, capsules, gelcaps, sachets, troches, sprays, beads (immediate or time release) and SECs (soft elastic capsules or "caplets"). In a preferred embodiment, formulations of the invention are administered orally

Other components of formulations include dyes, thickeners, plasticizers, flavoring agents, diluents, emulsifiers, disintegrants and binders. Disintegrants and binders include EMDEX, PRECIROL and AVICEL. The formulation can also include materials effective in protecting the biologically active substance from pH extremes of the stomach, or in releasing the nucleic acid over time, to optimize the delivery thereof to the gastrointestinal mucosa. Enteric coatings for acid-resistant tablets, capsules and caplets are known in the art and include cellulose acetate phthalate (CAP), propylene glycol, EUDRAGIT and sorbitan monoleate. Various methods for producing formulations with these components are well known in the art (see Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, PA, 1990).

The amount of biologically active substance administered will depend on the route of administration, the indication being treated as well as the individual being treated. For antisense oligonucleotides, the amount will range from about 0.01 $\mu$ g to 100g per kg body weight, several times per day to yearly.

Poly-L-lysine/alginate multiparticulate formulations are prepared according to techniques known by the skilled artisan. A particular general method is as follows. Sodium alginate is mixed with calcium chloride in water to form a calcium alginate pregel.

Poly-L-lysine and a biologically active substance (BAS) is mixed in water and then added to the pregel and mixed to form a multiparticulate suspension.

5 Alternatively, poly-L-lysine is added to the pregel thereby forming a multiparticulate suspension and subsequently adding BAS to the suspension.

PLGA multiparticulate formulations are prepared according to techniques well known to those skilled in the art. A particular general method is as follows. PLGA polymer and oil soluble components are dissolved in an organic solvent and water soluble components are dissolved in water. The biologically active substance (BAS) to be administered is dissolved in either the polymer solution or the aqueous solution as appropriate. The two solutions are combined and mixed thoroughly to give a dispersed phase. A continuous phase is prepared by dissolving a surfactant in a solvent such as water, mineral oil, heptane, octane, and cottonseed oil. A dispersion is then prepared by slowly adding the dispersed phase to the continuous phase while mixing. Temperature is then increased and volatile solvents are allowed to evaporate. The resulting multi-particulates may then be recovered by filtration from the solution.

25  
Example 1: poly-L-lysine/alginate particles  
with oligonucleotide ISIS-3521

30 formulation 1

Two solutions, one of sodium alginate (240mg), medium viscosity dissolved in approximately 350mL of distilled H<sub>2</sub>O and the other 52.96 mg of CaCl<sub>2</sub>·2H<sub>2</sub>O in approximately 50mL of dH<sub>2</sub>O were combined to give a calcium alginate pregel (0.06% alginate and 0.9mM calcium). 400mL of the calcium alginate pregel

was mixed with an 80mL solution of poly-L-lysine (PLL, 187.5mg, 7500mw) in dH<sub>2</sub>O. To the supernatant was added 240mg of oligo ISIS 3521 and stirred gently over 4 days. The resulting mixture proportions were 0.05% alginate, 0.75mM Ca, 52.08μM PLL (7500mw) and 0.05% oligonucleotide. Microparticles formed in the mixture were measured after 4 days of stirring by laser scattering on a Horiba LA-910 analyzer to determine mean particle size of 127.991μm (93.831 standard deviation).

After stirring, the 480mL microparticle mixture was equally divided into twelve 45mL (Falcon) tubes followed by centrifugaion for 30 minutes at 4000rpm. The amount of oligonucleotide associated with or bound to PLL-alginate particles was determined from a 4X dilution sample by UV absorbance at γ=260nm to be 176.41mg (73.5%). To purify the complex from gree oligo, 32.5mL of the clear supernatant was removed from each vial thus eliminating 51.67mg of unbound oligonucleotide. The remaining 7.5mL in each tube was then combined (90mL total) and filtered through a 0.2μ membrane filter under vacuum eliminating a further 6.62mg of unbound oligonucleotide. The remaining 40mL solution comprising 176.41mg (97.1%) bound oligo-microparticle complex and 5.3mg (2.9%) unbound oligo was then lyophilized for storage prior to biological testing.

#### Bioavailability

For in situ rat studies, 209.8mg of the lyophilized oligo microparticulate and 225.0mg of a lyophilized penetration enhancer mixture (PE) (sodium salts of CDCA, capric acid and lauric acid; 1:2:2) were combined with dH<sub>2</sub>O and vortexed to remove air bubbles and create a homogeneous paste. 1mL of the final

formulation (10mg oligo, 50mg PE) was administered by intrajejeunal installation into three rats (kind, weight, age, sex). Blood samples of 300µL were taken from femoral vein at time intervals of 30 minutes, 1, 2 and 3 hours post administration. 8µL EDTA was added to the samples and plasma harvested after centrifugation. Plasma levels of oligo ISIS-3521 were then determined by anion exchange HPLC, results shown in figure 1.

Example 2: PLGA particles with antisense oligonucleotide ISIS-2302

formulation 2a

0.2g of PLGA polymer was dissolved in 2mL methylene chloride (CH<sub>2</sub>Cl<sub>2</sub>) and 0.1g of oligo ISIS-2302 was dissolved in water (0.1mL) along with 0.2g of DMRIE (a 1:1 mixture of lipid 1,2-dimyristyloxypropyl-3-dimethyl-hydroxy ethyl ammonium bromide and cholesterol). The aqueous solution was added to the polymer solution to give a dispersed phase. A continuous phase was prepared by dissolving 0.5g of polyvinyl alcohol in 100mL of water. The dispersed phase was then slowly added to the continuous phase while mixing and continued mixing for about 2 hours and increasing the temperature to about 40°C to evaporate the volatile solvent.

formulation 2b

0.1g of PLGA polymer was dissolved in 1.0mL HFA (hexafluoroacetone) and 9.9mg of oligo ISIS-2302 was dissolved in water. The aqueous and polymer solutions were combined to give a dispersed phase. A continuous phase was prepared by dissolving 2.1g of sorbitan sesquioleate in 60mL of cottonseed oil. The dispersed

phase was then slowly added to the continuous phase while mixing and continued mixing for about 3 hours and increasing the temperature to about 50°C to evaporate the volatile solvent.

5

formulation 2c

0.2g of PLGA polymer was dissolved in a mixture of 1.5mL HFA and 0.5mL ACN (acetonitrile) and 20mg of oligo ISIS-2302 was dissolved in water. The aqueous and polymer solutions were combined to give a dispersed phase. A continuous phase was prepared by dissolving 6g of sorbitan sesquioleate in 200mL of cottonseed oil.

10

The dispersed phase was then slowly added to the continuous phase while mixing and continued mixing for about 1.5 hours at rt and increasing the temperature to 90°C to evaporate the volatile solvent.

15

formulation 2d

0.2g of PLGA polymer was dissolved in 1.5mL HFA and 22mg of the calcium salt of oligo ISIS-2302 was dissolved in water. The aqueous and polymer solutions were combined to give a dispersed phase. A continuous phase was prepared by dissolving 9g of sorbitan sesquioleate in 150mL of cottonseed oil. The dispersed phase was then slowly added to the continuous phase while mixing and continued mixing for about 2 hours and increasing the temperature to about 40°C to evaporate the volatile solvent.

20

25

30

Example 3:                    protamine particles with antisense  
                                 oligonucleotide ISIS-2302

Cationic protamine polymer was dissolved in water and mixed with an oligonucleotide solution comprising ISIS-2302 and a complex modifier in water.

35

The resulting precipitated particles were then separated by centrifugation or filtration. The specific modifier and relative amounts of the solution components are found in the table below.

5

Table I

protamine solution		oligo solution			
protamine (mg)	water (mL)	modifier	amount (mg)	water (mL)	oligo (mg)
10	1	none	-	0.5	5
90	1	Na-alginate	51	1	25
32 <sup>a</sup>	1	Na-alginate	19	1	10
6	1	trilysine	6	1	5
2.5-5	0.5	trilysine	3-6	0.5	5
0.6	0.1	CaCl <sub>2</sub>	3.5	0.2 <sup>b</sup>	1
53	1	bovine albumin	51	2	25
0.06	0.03	glucosamine	1-2	0.02	1
0.2	0.2	lysine	1	0.1	1
0.2	0.2	dilysine	1	0.1	1
0.2	0.2	trilysine	1	0.2	1
0.2	0.2	arginine	1	0.1	1
0.2	0.2	histidine	1	0.1	1
0.2	0.2	glucosamine	1	0.1	1
0.2	0.2	galactosamine	1	0.1	1
0.2	0.2	nicotinamide	1	0.1	1
0.2	0.2	creatine	1	0.1	1
0.4	0.02	arginine	2	0.2	1
0.125-1.0	0.025-0.2	none	-	0.1	0.5
0.5	0.1	lys ethyl ester	5.5	0.1	5
0.01-1.0	0.1	arg ethyl ester	10	0.11	1

<sup>a</sup> added CaCl<sub>2</sub> to complete precipitation

<sup>b</sup> CaCl<sub>2</sub> in 0.1mL water added to oligo in 0.1mL water



Example 4: chitosan, spermine and arginine-ethyl ester particles with antisense oligonucleotide ISIS-2302

Multiparticulate formulations comprising chitosan, spermine and arginine ethyl ester as carrier particles for ISIS-2302 were prepared by mixing a cationic particle-forming material with ISIS-2302 in water or saline. The specific components and amounts are as follows.

Table II

particle material	oligo solution
chitosan (0.125-1.0mg) in 0.05-0.4mL H <sub>2</sub> O	0.5mg in 0.1mL H <sub>2</sub> O
spermine (305mg) in 2mL PBS	296mg in 3.0mL PBS
arg-ethyl ester (10-500mg) in 1mL	5-50mg in 1mL H <sub>2</sub> O

**WHAT IS CLAIMED IS:**

1. A non-parenteral multi-particulate formulation comprising:
  - 5 a plurality of carrier particles;
  - a biologically active substance (BAS) to be delivered across a mucosal membrane; and
  - a penetration enhancer;wherein said biologically active substance is bound to  
10 said carrier particles.
2. The formulation according to claim 1, wherein said biologically active substance is an oligonucleotide.  
15
3. The formulation according to claim 2, wherein said oligonucleotide is an antisense oligonucleotide.
- 20 4. The formulation according to claim 3, wherein said oligonucleotide is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:7.
- 25 5. The formulation according to claim 1, wherein said carrier particles are bioadhesive.

6. The formulation according to claim 1,  
wherein said carrier particles comprise a particle-  
forming substance selected from the group consisting of  
poly-amino acids; polyimines; polyacrylates;  
5 polyalkylacrylates, polyoxethanes,  
polyalkylcyanoacrylates; cationized gelatins, albumins,  
starches, acrylates, polyethyleneglycols (PEG) and  
starches; polyalkylcyanoacrylates; DEAE-derivatized  
polyimines, pollulans, celluloses and starches.

10 7. The formulation according to claim 1,  
wherein said carrier particles comprise a particle-  
forming material selected from the group consisting of  
chitosan, poly-L-lysine, polyhistidine, polyornithine,  
15 polyspermines, protamine, polyvinylpyridine,  
polythiodiethylamino-methylethylene P(TDAE),  
polyaminostyrene (e.g. p-amino),  
poly(methylcyanoacrylate), poly(ethylcyanoacrylate),  
poly(butylcyanoacrylate), poly(isobutylcyanoacrylate),  
20 poly(isohexylcynaoacrylate), DEAE-methacrylate, DEAE-  
hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-  
dextran, polymethylacrylate, polyhexylacrylate,  
poly(D,L-lactic acid), poly(DL-lactic-co-glycolic acid  
(PLGA), and polyethyleneglycol (PEG).

25 8. The formulation according to claim 1,  
wherein said carrier particles are polycationic.

9. The formulation according to claim 8,  
30 wherein said carrier particles comprise a complex of  
poly-L-lysine and alginate; or protamine and alginate,  
lysine, dilysine, trilysine, calcium, albumin,  
glucosamine, arginine, galactosamine, nicotinamide,  
creatine, lysine-ethyl ester and arginine-ethyl ester.

35

10. The formulation according to claim 1,  
wherein said carrier particles are other than  
polycationic.

5 11. The formulation according to claim 10,  
wherein said carrier particles comprise poly(DL-lactic-  
co-glycolic acid) (PLGA).

10 12. The formulation according to claim 1,  
wherein said carrier particles are miniparticles.

13. The formulation according to claim 1,  
wherein said carrier particles are microparticles.

15 14. The formulation according to claim 1,  
wherein said carrier particles are nanoparticles.

20 15. The formulation according to claim 1,  
wherein said penetration enhancer is selected from the  
group consisting of surfactants, fatty acids/salts,  
bile acids/salts, chelating agents and non-chelating  
non-surfactant penetration enhancers.

25 16. The formulation according to claim 15,  
wherein said penetration enhancer is selected from the  
group consisting of fatty acids, bile acids and salts  
and mixtures thereof.

30 17. The formulation according to claim 15,  
wherein said penetration enhancer is selected from  
UDCA, CDCA and salts and mixtures thereof.

18. The formulation according to claim 15,  
wherein said penetration enhancer is a mixture  
comprising the sodium salts of UDCA, capric acid and  
5 lauric acid.

19. The formulation according to claim 1,  
wherein said penetration enhancer is a component of  
said particle.  
10

20. The formulation according to claim 1,  
wherein the surface of said carrier particle is  
substantially coated with said penetration enhancer.

15 21. The formulation according to claim 1,  
further comprising a mucolytic material.

22. The formulation according to claim 21,  
wherein said mucolytic material is selected from the  
20 group consisting of N-acetylcysteine, dithiothreitol,  
pepsin, pilocarpine, guaifenesin, glyceryl guaiacolate,  
terpin hydrate, ammonium chloride, guattenesin,  
ambroxol, bromhexine, carbocysteine, domiodol,  
letosteine, mecysteine, mesna, sobrerol, stepronin,  
25 tiopronin and tyloxapol.

23. The formulation according to claim 1 in a  
dosage form selected from the group consisting of  
tablets, capsules and filled gelcaps.  
30

24. The formulation according to claim 23,  
further comprising an enteric material protecting the  
dosage form from degradation in a gastric environment.

25. The formulation according to claim 24,  
wherein said enteric material is selected from the  
group consisting of cellulose acetate phthalate (CAP),  
propylene glycol, EUDRAGIT and sorbitan monoleate.

5

26. The formulation according to claim 24,  
wherein said enteric material substantially coats the  
outer surface of the dosage form.

10

27. The formulation according to claim 24,  
wherein said enteric material substantially coats the  
outer surface of the individual carrier particles.

15

28. An oral formulation, comprising  
a plurality of carrier particles; and  
a biologically active substance (BAS) to be  
delivered across a mucosal membrane, wherein said  
biologically active substance is bound to said carrier  
particles.

20

29. The oral formulation according to claim 28,  
wherein said biologically active substance is an  
oligonucleotide.

25

30. The oral formulation according to claim 28,  
further comprising a penetration enhancer.

30

31. A method of delivering a biologically  
active substance across a mucosal membrane, comprising  
introducing to the mucosal membrane a multi-particulate  
formulation according to claim 1.

32. The method according to claim 31, wherein  
said biologically active substance is an  
oligonucleotide and said formulation is administered  
5 orally to a mammal.

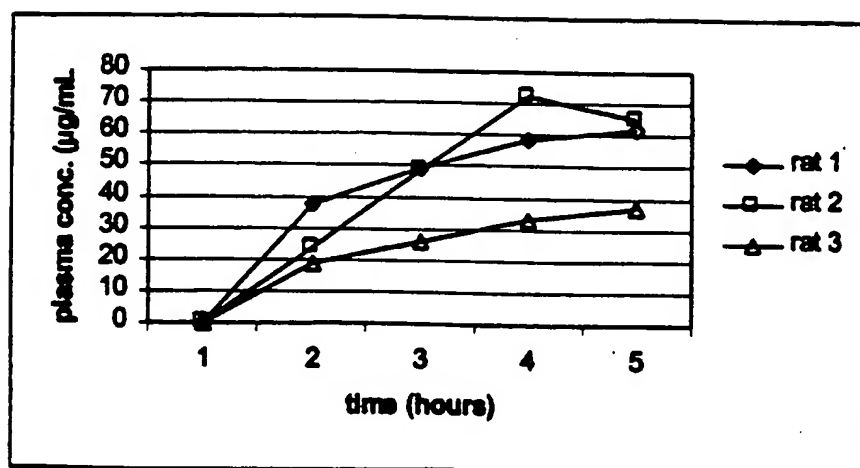


FIGURE 1



## SEQUENCE LISTING

<110> HARDEE, Gregory E  
TILLMAN, Lloyd G  
GONZALES-FERREIRO, Maria  
MEHTA, Rahul C  
TENG, Ching-Leou

<120> MULTIPARTICULATE FORMULATION

<130> 049202/2001

<140> 09/256,515

<141> 1999-02-23

<160> 9

<170> PatentIn Ver. 2.1

<210> 1

<211> 20

<212> DNA

<213> Artificial Sequence

<400> 1

tcccgctgt gacatgcatt

20

<210> 2

<211> 20

<212> DNA

<213> Artificial Sequence

<400> 2

gccaagctg gcatccgtca

20

<210> 3

<211> 21

<212> DNA

<213> Artificial Sequence

<400> 3

gcgtttgctc ttcttcttgc g

21

<210> 4

<211> 20  
<212> DNA  
<213> Artificial Sequence

<400> 4  
gttctcgctg gtgagtttca  
20

<210> 5  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<400> 5  
tccgtcatcg ctccctcaggg  
20

<210> 6  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<400> 6  
gcgtttgctc ttcttcttgc g  
21

<210> 7  
<211> 8  
<212> DNA  
<213> Artificial Sequence

<400> 7  
ttgggggtt  
8

<210> 8  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<400> 8  
gtgctcatgg tgcacggtct  
20

<210> 9

WO 00/50050

PCT/US00/04662

<211> 20

<212> DNA

<213> Artificial Sequence

<400> 9

gtgtgccaga caccctatct

20

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/04662

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : A61K 35/64; A61K 48/00; C12Q 1/68; CO7H 21/02, 21/04

US CL : 424/ 93.1, 450; 435/6; 514/44; 536/23.1, 24.3, 24.31, 24.5

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/ 93.1, 450; 435/6; 514/44; 536/23.1, 24.3, 24.31, 24.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Continuation Sheet**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,795,587 A (GAO et al) 18 August 1998 (18.08.1998), col. 7, lines 20-67.	1-3, 5-16, 19-20, 28-32
X --- Y	US 5,783,567 A (HEDLEY et al) 21 July 1998 (21.07.1998), col. 10-11.	17-18, 21-27 1-3, 5-16, 19-20, 28-32
X	WO 98-49348 A1 (ISIS PHARMACEUTICALS, INC.) 5 November 1998 (5.11.1998), pages 19-23.	17-18, 21-27 1-3, 5-32

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

12 April 2000 (12.04.2000)

Date of mailing of the international search report

06 JUN 2000

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703)305-3230

Authorized officer

George Elliott

Telephone No. 703-308-0196

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/04662

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
The Sequence Listing in computer readable form submitted for this application is defective. Claim 4 is therefore unsearchable since this claim recites specific sequence identification numbers (SEQ ID NO).
2. ☐ Claim Nos.: 4  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐  
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/04662

**Continuation of B. FIELDS SEARCHED Item 3: STN-CAPLUS, USPAT, EPO, JPO, DERWENT**  
search terms: penetration enhancer, carrier particle, multi-particulate formulation, antisense, oligonucleotide, polynucleotide, bioadhesive, polycationic, liposomes